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Dear Sirs

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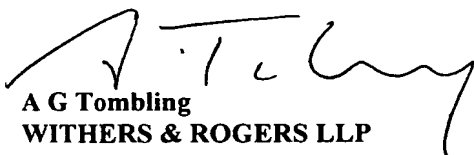
28. Sep. 2005

**European Patent Application No. 04759056.7
based on PCT/US2004/009704
Breast Endothelial Cell Expression Patterns
Genzyme Corporation, The Johns Hopkins University**

I enclose EPO form 1200 entering the above-identified application into the European regional phase. An amended set of claims is enclosed. The claims have been amended by correcting a typographical error in claim 1. In particular, the word "brain" has been corrected to "breast" in line 2 of claim 1. Claims 17 to 20 have also been amended so that they correctly depend from claim 15. Finally, claims 14 and 36 have been amended to place them into the second medical use format.

The official fees in respect of this application are being paid separately to Cash & Accounts.

Yours faithfully



**A G Tombling
WITHERS & ROGERS LLP**

Enc: Form 1200
Claims

Rel. 3 (2005) LLP

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CLAIMS:

1. A method to aid in diagnosing breast tumor, comprising the steps of:
detecting an expression product of at least one gene in a first brain tissue sample suspected of being neoplastic wherein said at least one gene is selected from the group consisting of hypothetical protein DKFZp434G171; heat shock 70kDa protein 1A; jagged 1 (Alagille syndrome); cyclin-dependent kinase 3; 6-phosphogluconolactonase; likely homolog of rat and mouse retinoid-inducible serine carboxypeptidase; plasmalemma vesicle associated protein; NADH:ubiquinone oxidoreductase MLRQ subunit homolog; HIF-1 responsive RTP801; ribosomal protein L27; secreted protein, acidic, cysteine-rich (osteonectin); hexokinase 1; ribosomal protein L13a; collagen, type IV, alpha 1; insulin-like growth factor binding protein 7; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); heat shock 10kDa protein 1 (chaperonin 10); calcium channel, voltage-dependent, alpha 1H subunit; CD9 antigen (p24); TEM17; TEM13, Thy-1 cell surface antigen; Tax interaction protein 1; dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive); hypothetical protein MGC34648; putative translation initiation factor; insulin-like growth factor binding protein 4; matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase); heterogeneous nuclear ribonucleoprotein R; bHLH factor Hes4; collagen, type VI, alpha 2; T-box 2; glyceraldehyde-3-phosphate dehydrogenase; G protein-coupled receptor 4; collagen, type I, alpha 1; ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1); ribosomal protein, large, P1; TEM10, COL1A2 involved in tissue remodeling; heat shock 70kDa protein 8; KIAA0152 gene product; Ca²⁺-promoted Ras inactivator; serine/arginine repetitive matrix 2; hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor); benzodiazapine receptor (peripheral); ectonucleoside triphosphate diphosphohydrolase 1; heparan sulfate proteoglycan 2 (perlecan); fibromodulin; hairy/enhancer-of-split related with YRPW motif 1; collagen, type V, alpha 3;

hairy/enhancer-of-split related with YRPW motif-like; hypothetical protein MGC2731; amino-terminal enhancer of split; mitogen-activated protein kinase 9; regulator of G-protein signalling 5; prothymosin, alpha (gene sequence 28); tubulin, beta, 2; protease, serine, 23; hypothetical protein FLJ20898; calpain 1, (mu/I) large subunit; interferon, alpha-inducible protein (clone IFI-6-16); ESTs, Weakly similar to T25031 hypothetical protein T20D3.3 - *Caenorhabditis elegans* [C.elegans]; major histocompatibility complex, class I, C; hypoxia up-regulated 1; complement component 4B; prefoldin 2; cytoskeleton-associated protein 1; Rho GTPase activating protein 4; Homo sapiens clone FLC1492 PRO3121 mRNA, complete cds; transducin-like enhancer of split 2 (E(sp1) homolog, *Drosophila*); ribosomal protein L37; hypothetical protein MGC4677; ESTs, Highly similar to MT1A_HUMAN METALLOTHIONEIN-IA (MT-1A) [H.sapiens]; TEM11, nidogen (enactin); guanine nucleotide binding protein (G protein), gamma 5; matrix Gla protein; heat shock 105kD; GNAS complex locus; Homo sapiens cDNA FLJ11658 fis, clone HEMBA1004577; H19, imprinted maternally expressed untranslated mR NA; protein tyrosine phosphatase type IVA, member 3; snail homolog 1 (*Drosophila*); integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II); tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); peptidylprolyl isomerase B (cyclophilin B); MARCKS-like protein; FAST kinase; protease, serine, 11 (IGF binding); beta-2-microglobulin; delta sleep inducing peptide, immunoreactor; collagen, type IV, alpha 2; immediate early response 3; cadherin 5, type 2, VE-cadherin (vascular epithelium); RGC32 protein; guanylate cyclase 1, soluble, beta 3; major histocompatibility complex, class I, B; ribonuclease, RNase A family, 1 (pancreatic); collagen, type XVIII, alpha 1; v-jun sarcoma virus 17 oncogene homolog (avian); Homo sapiens mRNA; cDNA DKFZp686G1610 (from clone DKFZp686G1610); nucleolin; lectin, galactoside-binding, soluble, 3 binding protein; Lysosomal-associated multispinning membrane protein-5; ribosomal protein S16; guanine nucleotide binding protein (G protein), gamma 12; serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1; biglycan; DnaJ (Hsp40) homolog, subfamily B, member 1; tumor

rejection antigen (gp96) 1; interferon, alpha-inducible protein (clone IFI-15K); solute carrier family 21 (prostaglandin transporter), member 2; CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated); serum/glucocorticoid regulated kinase; mitogen-activated protein kinase; receptor (calcitonin) activity modifying protein 3; sema domain, immunoglobulin domain (Ig); benzodiazapine receptor (peripheral) – mitochondrial; C1 domain-containing phosphatase & tensin-like; and Notch homolog 3 (Drosophila);

and

comparing expression of the at least one gene in the first breast tissue sample with expression of the at least one gene in a second breast tissue sample which is normal, wherein increased expression of the at least one gene in the first breast tissue sample relative to the second tissue sample identifies the first breast tissue sample as likely to be neoplastic.

2. The method of claim 1 wherein the increased expression of the at least one gene in the first breast tissue sample relative to the second tissue sample is at least two-fold higher.
3. The method of claim 1 wherein the increased expression of the at least one gene in the first breast tissue sample relative to the second tissue sample is at least five-fold higher.
4. The method of claim 1 wherein the increased expression of the at least one gene in the first breast tissue sample relative to the second tissue sample is at least ten-fold higher.
5. The method of claim 1 wherein the expression product is RNA.
6. The method of claim 1 wherein the expression product is protein.

7. The method of claim 1 wherein the first and second tissue samples are from a human.
8. The method of claim 1 wherein the first and second tissue samples are from the same human.
9. The method of claim 1 wherein the step of detecting is performed using a Western blot.
10. The method of claim 1 wherein the step of detecting is performed using an immunoassay.
11. The method of claim 1 wherein the step of detecting is performed using an immunohistochemical assay.
12. The method of claim 1 wherein the step of detecting is performed using SAGE.
13. The method of claim 1 wherein the step of detecting is performed using hybridization to a microarray.
14. Use of an antibody, wherein the antibody specifically binds to an extracellular epitope of a protein selected from the group consisting of benzodiazapine receptor (peripheral); cadherin 5, type 2, VE-cadherin (vascular epithelium); calcium channel, voltage-dependent, alpha 1H subunit; CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated); CD9 antigen (p24); dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive); ectonucleoside triphosphate diphosphohydrolase 1; G protein-coupled receptor 4; hypothetical protein FLJ20898; hypoxia up-regulated 1; immediate early response 3; interferon, alpha-inducible protein (clone IFI-6-16); jagged 1 (Alagille syndrome); KIAA0152 gene product; Lysosomal-associated multispanning membrane protein-5; major histocompatibility complex, class I, B; major histocompatibility complex, class I, C; NADH:ubiquinone oxidoreductase

MLRQ subunit homolog; Notch homolog 3 (Drosophila); plasmalemma vesicle associated protein; solute carrier family 21 (prostaglandin transporter), member 2; TEM13, Thy-1 cell surface antigen; receptor (calcitonin) activity modifying protein 3; sema domain, immunoglobulin domain (Ig); benzodiazapine receptor (peripheral) – mitochondrial; and TEM17; in the manufacture of a medicament for treating a breast tumor, whereby immune destruction of cells of the breast tumor is triggered.

15. The use of claim 14 wherein the antibody is conjugated to a diagnostic or therapeutic reagent.

16. The use of claim 14 wherein the breast tumor is multidrug-sensitive.

17. The use of claim 14 wherein the reagent is a chemotherapeutic agent.

18. The use of claim 14 wherein the reagent is a cytotoxin.

19. The use of claim 14 wherein the reagent is a non-radioactive label.

20. The use of claim 14 wherein the reagent is a radioactive compound.

21. The use of claim 14 wherein the breast tumor is in a human.

22. A method of identifying a test compound as a potential anti-cancer or anti-breast tumor drug, comprising the step of:

contacting a test compound with a cell which expresses at least one gene selected from the group consisting of hypothetical protein DKFZp434G171; heat shock 70kDa protein 1A; jagged 1 (Alagille syndrome); cyclin-dependent kinase 3; 6-phosphogluconolactonase; likely homolog of rat and mouse retinoid-inducible serine carboxypeptidase; plasmalemma vesicle associated protein; NADH:ubiquinone oxidoreductase MLRQ subunit homolog; HIF-1 responsive RTP801; ribosomal protein L27; secreted protein, acidic, cysteine-rich (osteonectin); hexokinase 1; ribosomal protein

L13a; collagen, type IV, alpha 1; insulin-like growth factor binding protein 7; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); heat shock 10kDa protein 1 (chaperonin 10); calcium channel, voltage-dependent, alpha 1H subunit; CD9 antigen (p24); TEM17; TEM13, Thy-1 cell surface antigen; Tax interaction protein 1; dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive); hypothetical protein MGC34648; putative translation initiation factor; insulin-like growth factor binding protein 4; matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase); heterogeneous nuclear ribonucleoprotein R; bHLH factor Hes4; collagen, type VI, alpha 2; T-box 2; glyceraldehyde-3-phosphate dehydrogenase; G protein-coupled receptor 4; collagen, type I, alpha 1; ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1); ribosomal protein, large, P1; TEM10, COL1A2 involved in tissue remodeling; heat shock 70kDa protein 8; KIAA0152 gene product; Ca²⁺-promoted Ras inactivator; serine/arginine repetitive matrix 2; hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor); benzodiazepine receptor (peripheral); ectonucleoside triphosphate diphosphohydrolase 1; heparan sulfate proteoglycan 2 (perlecan); fibromodulin; hairy/enhancer-of-split related with YRPW motif 1; collagen, type V, alpha 3; hairy/enhancer-of-split related with YRPW motif-like; hypothetical protein MGC2731; amino-terminal enhancer of split; mitogen-activated protein kinase 9; regulator of G-protein signalling 5; prothymosin, alpha (gene sequence 28); tubulin, beta, 2; protease, serine, 23; hypothetical protein FLJ20898; calpain 1, (mu/I) large subunit; interferon, alpha-inducible protein (clone IFI-6-16); ESTs, Weakly similar to T25031 hypothetical protein T20D3.3 - *Caenorhabditis elegans* [*C.elegans*]; major histocompatibility complex, class I, C; hypoxia up-regulated 1; complement component 4B; prefoldin 2; cytoskeleton-associated protein 1; Rho GTPase activating protein 4; Homo sapiens clone FLC1492 PRO3121 mRNA, complete cds; transducin-like enhancer of split 2 (E(sp1) homolog, *Drosophila*); ribosomal protein L37; hypothetical protein MGC4677; ESTs, Highly similar to MT1A_HUMAN METALLOTHIONEIN-1A (MT-1A) [*H.sapiens*]; TEM11, nidogen (enactin); guanine nucleotide binding protein (G protein), gamma 5; matrix Gla protein; heat shock 105kD; GNAS complex locus; Homo sapiens cDNA FLJ11658 fis, clone HEMBA1004577; H19, imprinted maternally expressed untranslated mR NA; protein tyrosine phosphatase type

IVA, member 3; snail homolog 1 (Drosophila); integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II); tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); peptidylprolyl isomerase B (cyclophilin B); MARCKS-like protein; FAST kinase; protease, serine, 11 (IGF binding); beta-2-microglobulin; delta sleep inducing peptide, immunoreactor; collagen, type IV, alpha 2; immediate early response 3; cadherin 5, type 2, VE-cadherin (vascular epithelium); RGC32 protein; guanylate cyclase 1, soluble, beta 3; major histocompatibility complex, class I, B; ribonuclease, RNase A family, 1 (pancreatic); collagen, type XVIII, alpha 1; v-jun sarcoma virus 17 oncogene homolog (avian); Homo sapiens mRNA; cDNA DKFZp686G1610 (from clone DKFZp686G1610); nucleolin; lectin, galactoside-binding, soluble, 3 binding protein; Lysosomal-associated multispinning membrane protein-5; ribosomal protein S16; guanine nucleotide binding protein (G protein), gamma 12; serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1; biglycan; DnaJ (Hsp40) homolog, subfamily B, member 1; tumor rejection antigen (gp96) 1; interferon, alpha-inducible protein (clone IFI-15K); solute carrier family 21 (prostaglandin transporter), member 2; CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated); serum/glucocorticoid regulated kinase; mitogen-activated protein kinase; receptor (calcitonin) activity modifying protein 3; sema domain, immunoglobulin domain (Ig); benzodiazapine receptor (peripheral) – mitochondrial; C1 domain-containing phosphatase & tensin-like; and Notch homolog 3 (Drosophila);

monitoring an expression product of the at least one gene; and

identifying the test compound as a potential anti-cancer drug if it decreases the expression of the at least one gene.

23. The method of claim 22 wherein the cell is a human cell.
24. The method of claim 22 wherein the cell is a breast tumor cell.
25. The method of claim 22 wherein the cell is a human breast tumor cell.

26. The method of claim 22 wherein the expression product is RNA.
27. The method of claim 22 wherein the expression product is protein.
28. The method of claim 22 wherein the cell overexpresses the at least one gene relative to a normal cell of the same tissue.
29. The method of claim 22 wherein expression of at least two of said genes is monitored.
30. The method of claim 22 wherein expression of at least three of said genes is monitored.
31. The method of claim 22 wherein expression of at least four of said genes is monitored.
32. The method of claim 22 wherein the test compound is identified if the decrease in expression is at least two-fold.
33. The method of claim 22 wherein the test compound is identified if the decrease in expression is at least five-fold.
34. The method of claim 22 wherein the decrease in expression is at least ten-fold.
35. The method of claim 22 wherein the test compound is identified as an anti-breast tumor drug.
36. Use of a protein or nucleic acid encoding a protein selected from the group consisting of: hypothetical protein DKFZp434G171; heat shock 70kDa protein 1A; jagged 1 (Alagille syndrome); cyclin-dependent kinase 3; 6-phosphogluconolactonase; likely homolog of rat and mouse retinoid-inducible serine carboxypeptidase;

plasmalemma vesicle associated protein; NADH:ubiquinone oxidoreductase MLRQ subunit homolog; HIF-1 responsive RTP801; ribosomal protein L27; secreted protein, acidic, cysteine-rich (osteonectin); hexokinase 1; ribosomal protein L13a; collagen, type IV, alpha 1; insulin-like growth factor binding protein 7; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); heat shock 10kDa protein 1 (chaperonin 10); calcium channel, voltage-dependent, alpha 1H subunit; CD9 antigen (p24); TEM17; TEM13, Thy-1 cell surface antigen; Tax interaction protein 1; dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive); hypothetical protein MGC34648; putative translation initiation factor; insulin-like growth factor binding protein 4; matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase); heterogeneous nuclear ribonucleoprotein R; bHLH factor Hes4; collagen, type VI, alpha 2; T-box 2; glyceraldehyde-3-phosphate dehydrogenase; G protein-coupled receptor 4; collagen, type I, alpha 1; ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1); ribosomal protein, large, P1; TEM10, COL1A2 involved in tissue remodeling; heat shock 70kDa protein 8; KIAA0152 gene product; Ca²⁺-promoted Ras inactivator; serine/arginine repetitive matrix 2; hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor); benzodiazapine receptor (peripheral); ectonucleoside triphosphate diphosphohydrolase 1; heparan sulfate proteoglycan 2 (perlecan); fibromodulin; hairy/enhancer-of-split related with YRPW motif 1; collagen, type V, alpha 3; hairy/enhancer-of-split related with YRPW motif-like; hypothetical protein MGC2731; amino-terminal enhancer of split; mitogen-activated protein kinase 9; regulator of G-protein signalling 5; prothymosin, alpha (gene sequence 28); tubulin, beta, 2; protease, serine, 23; hypothetical protein FLJ20898; calpain 1, (mu/I) large subunit; interferon, alpha-inducible protein (clone IFI-6-16); ESTs, Weakly similar to T25031 hypothetical protein T20D3.3 - *Caenorhabditis elegans* [*C.elegans*]; major histocompatibility complex, class I, C; hypoxia up-regulated 1; complement component 4B; prefoldin 2; cytoskeleton-associated protein 1; Rho GTPase activating protein 4; Homo sapiens clone FLC1492 PRO3121 mRNA, complete cds; transducin-like enhancer of split 2 (E(spl) homolog, *Drosophila*); ribosomal protein L37; hypothetical protein MGC4677; ESTs, Highly similar to MT1A_HUMAN METALLOTHIONEIN-1A (MT-1A) [*H.sapiens*]; TEM11, nidogen (enactin); guanine

nucleotide binding protein (G protein), gamma 5; matrix Gla protein; heat shock 105kD; GNAS complex locus; Homo sapiens cDNA FLJ11658 fis, clone HEMBA1004577; H19, imprinted maternally expressed untranslated mRNA; protein tyrosine phosphatase type IVA, member 3; snail homolog 1 (Drosophila); integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II); tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); peptidylprolyl isomerase B (cyclophilin B); MARCKS-like protein; FAST kinase; protease, serine, 11 (IGF binding); beta-2-microglobulin; delta sleep inducing peptide, immunoreactor; collagen, type IV, alpha 2; immediate early response 3; cadherin 5, type 2, VE-cadherin (vascular epithelium); RGC32 protein; guanylate cyclase 1, soluble, beta 3; major histocompatibility complex, class I, B; ribonuclease, RNase A family, 1 (pancreatic); collagen, type XVIII, alpha 1; v-jun sarcoma virus 17 oncogene homolog (avian); Homo sapiens mRNA; cDNA DKFZp686G1610 (from clone DKFZp686G1610); nucleolin; lectin, galactoside-binding, soluble, 3 binding protein; Lysosomal-associated multispanning membrane protein-5; ribosomal protein S16; guanine nucleotide binding protein (G protein), gamma 12; serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1; biglycan; DnaJ (Hsp40) homolog, subfamily B, member 1; tumor rejection antigen (gp96) 1; interferon, alpha-inducible protein (clone IFI-15K); solute carrier family 21 (prostaglandin transporter), member 2; CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated); serum/glucocorticoid regulated kinase; mitogen-activated protein kinase; receptor (calcitonin) activity modifying protein 3; sema domain, immunoglobulin domain (Ig); benzodiazepine receptor (peripheral) – mitochondrial; C1 domain-containing phosphatase & tensin-like; and Notch homolog 3 (Drosophila), in the manufacture of a medicament for inducing an immune response to a breast tumor, whereby an immune response to the protein is induced.

37. The use of claim 36 wherein a protein is used in the manufacture of a medicament.

38. The use of claim 36 wherein a nucleic acid is used in the manufacture of a medicament.
39. The use of claim 38 wherein the medicament is for administration intramuscularly.
40. The use of claim 36 wherein the medicament comprises an immune adjuvant.
41. The use of claim 36 wherein the medicament is for inducing an immune response to a breast tumor in a mammal having a breast tumor.
42. The use of claim 36 wherein the medicament is for inducing an immune response to a breast tumor in a mammal that has had a breast tumor surgically removed.